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ESSENTIAL GUIDES TO METHOD DEVELOPMENT IN THIN-LAYER (PLANAR) CHROMATOGRAPHY

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Introduction

One of the most critical steps of qualitative and quantitative planar (thin-layer) chromatographic (TLC) analysis is development of a method resulting in sufficient separation. The main steps of method development are summarized in **Figure 1**. The first stage is selection of the stationary phase, the vapour phase, and suitable solvents. This stage is the *sine qua non* of method development, and the selection of these can occasionally immediately result in a suitable separation. For most real separation problems the second stage, optimization of the mobile phase is also necessary. The third part of method development is selection of the final conditions, for example the mode of development, transfer of the mobile phase to an appropriate forced-flow method, and last but not least, the selection of suitable operating parameters. This paper gives essential guides to method development in planar chromatography and draws attention to the most important considerations.

Stationary Phase Selection

TLC separations can be performed on modified, unmodified, and impregnated stationary phases, because of differences between the chemical properties of the sorbent material and those of compounds present in the sample to be separated. Different types of chromatographic process (normal-phase, reversed-phase, partition, and ion exchange chromatography) can be distinguished on the basis of the types of interactions involved. Although more than 90% of TLC separations are performed on silica, chemically bonded phases have recently become increasingly popular for solving special separation problems.

In normal-phase chromatography the hydroxyl groups on the surface of the silica are the polar, active centres which result in the interactions leading to the retention of the compounds to be separated. These interactions are mainly hydrogen-bonding and induced dipole–dipole interactions. The stationary phase can generally be characterized in terms of its specific surface area, specific pore volume, and mean pore diameter.

Unmodified stationary phases include silicas, aluminas, kieselguhr, silicates, controlled-porosity glass, cellulose, starch, gypsum, polyamides, and

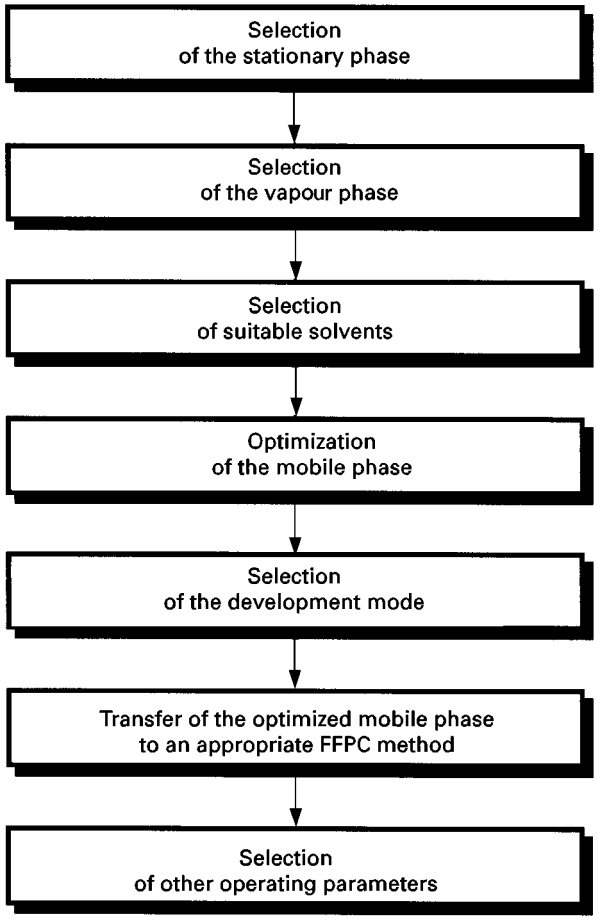
Selection of the stationary phase
Selection of the vapour phase
Selection of suitable solvents
Optimization of the mobile phase
Selection of the development mode
Transfer of the optimized mobile phase to an appropriate FFPC method
Selection of other operating parameters

Figure 1 Schematic diagram of method development.

chitin. For TLC separations silica is manufactured by spontaneous polymerization and dehydration of aqueous silicic acid, which is prepared by adding acid to a solution of sodium silicate. The product of this process is an amorphous, porous solid, the specific surface area of which can vary over a wide range (200 to more than 100 $m^2 g^{-1}$, as can the average pore diameter (10–1500 Å).

Modified silicas can be nonpolar or polar adsorbents. The former include silicas bearing alkane or alkene chains or phenyl groups, whereas the polar modified silicas contain cyano, diol, amino, or thiol groups or substance-specific complexing ligands. The structures of some chemically modified silicas are shown in **Figure 2**.

Almost all the stationary phases used in normal- and reversed-phase column liquid chromatography are also available for TLC. The dimensions of commercially available analytical thin-layer plates are 10×10, 10×20 or 20×20 cm; the layer thickness is 20 or 25 μm. It is generally accepted that better resolution is obtained on thinner layers (10 μm), depending on the mode of detection. The silica materials commonly used for precoated plates have an average particle size of ca. 11 μm, the size range is from 3 to 18 μm; for analytical layers prepared in the user's laboratory the average particle size is 15 μm and the range of particle sizes is much greater. The average particle size of precoated high-performance TLC (HPTLC) plates is now 5–6 μm and the range of particle sizes is very small.

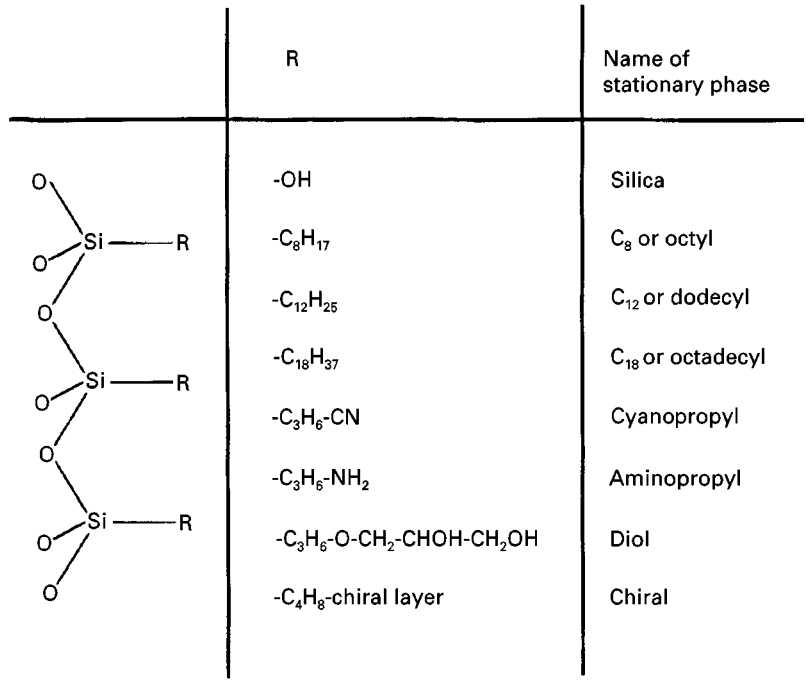

Figure 2 The structures of some commercially available surface-modified silicas.

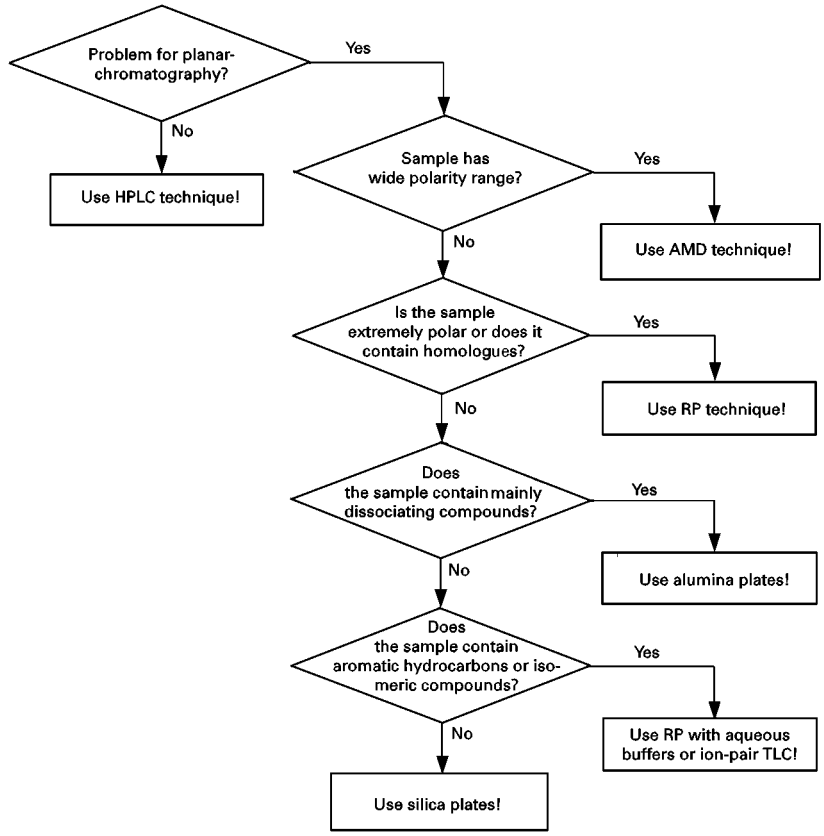

Figure 3 Flow chart illustrating a systematic approach for the selection of the appropriate separation technique and stationary phase.

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Precoated analytical layers with a preadsorbent zone are also commercially available for linear development. This zone serves to hold the sample until development begins. Compounds soluble in the solvent system pass through the preadsorbent zone and are concentrated in a narrow band on entering the chromatographic layer; this improves resolution. **Figure 3** gives a decision flow chart for the systematic selection of the appropriate separation technique and stationary phase.

Vapour Phase Selection

In planar chromatography the separation process occurs in a three-phase system of stationary, mobile, and vapour phases, all of which interact both with each other and with the operating conditions. Selection of chamber type and vapour space is a variable offered only by planar chromatography as the third dimension of the chromatographic parameters. The role of the vapour phase in TLC is well known, although little attention is given to this in practice.

In planar chromatography two basic types of chromatographic chamber must be distinguished. In the common normal (N) chamber the distance between the layer and the wall of the chromatographic tank is more than 3 mm. If this distance is smaller, the chamber is said to have the S configuration. Both types of chamber can be used for unsaturated or saturated systems. As a rule of thumb, if the sample contains fewer than seven compounds to be quantitatively determined, saturated N chambers must be selected for method development. If the sample contains more than seven substances, or the separation is very difficult, S chambers must be selected which enable transfer of the optimized mobile phase by forced-flow.

Often the separation problem cannot be solved by use of conventional TLC with solvent migration by capillary action, because of the relatively modest separating power of the method. In such circumstances use of one of the different forced-flow techniques is necessary; this must be considered during selection of the vapour phase. The chambers used for forced-flow planar separations can be also assigned to the above two categories. The chambers used for over-pressured layer chromatography (OPLC) are unsaturated S chambers, theoretically and practically devoid of any vapour space. This must be considered in the selection of appropriate solvents and during the

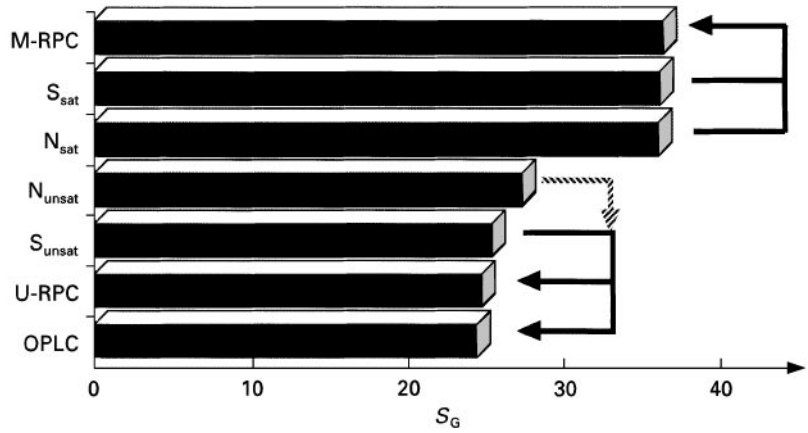

Figure 4 The saturation grade of different forced-flow methods, in comparison with the N and S chambers.

optimization of the solvent system. In rotation planar chromatography (RPC) the size, and thus the extent of saturation, of the vapour phase can be varied. In RPC the micro and ultramicro chambers belong to the S-chamber type. Because in microchamber RPC the plate rotates with the small chromatographic chamber, and the distance between the layer and the lid of the chamber is less than 2 mm, the vapour space is rapidly saturated. In ultramicrochamber RPC the lid of the chamber is placed directly on the plate and so in practice there is no vapour space, as in OPLC.

When a mobile phase is transferred from a chromatographic tank separation to a forced-flow technique, the vapour phase can be characterized on the basis of the saturation grade (S_G). The S_G value of a given chromatographic chamber can be calculated by dividing the sum of the hR_F values of the three furthest-migrating substances by the sum of the hR_F values of all the components, subtracting the result from 1, and multiplying the answer by 100. The saturation grade can be used as a measure of the reproducibility of separations with given stationary and mobile phases and at different temperatures and humidity; this enables transfer of the mobile phase to other vapour-phase conditions. **Figure 4** shows the saturation grade of the different chromatographic chambers. The lines indicate suggested mobile phase transfer possibilities; the dotted line indicates other mobile phases which might be used, but with less probability of success.

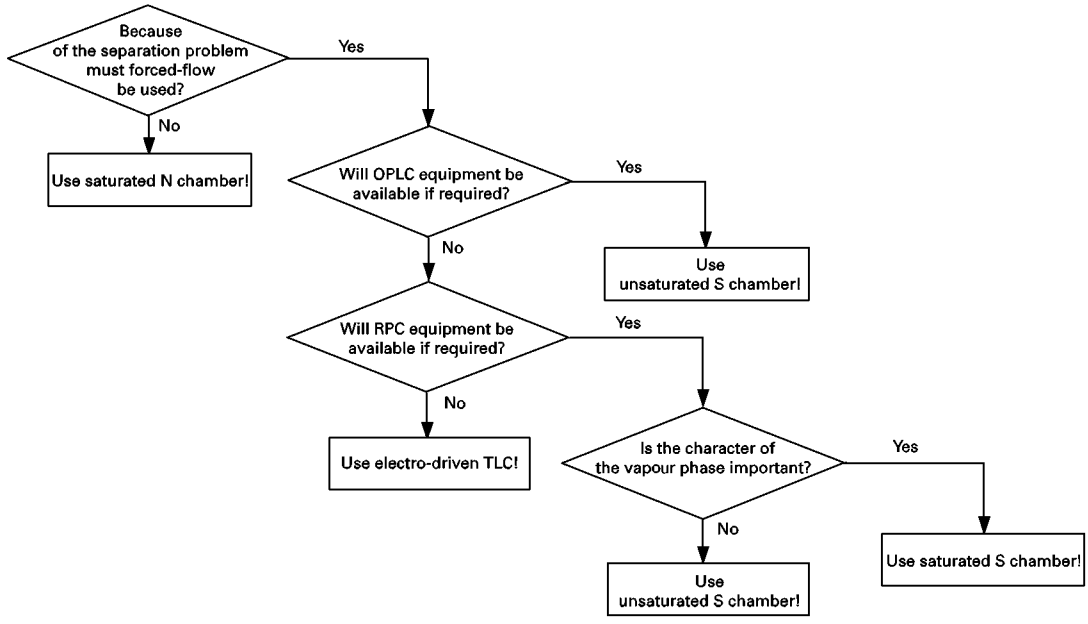

Figure 5 Flow chart illustrating a systematic approach for the selection of the appropriate chromatographic chamber and vapour phase.

Among the forced-flow methods the highest separating power is obtained with OPLC, because of the optimum mobile phase velocity on the HPTLC plate and the greater separation distance. If, therefore, the quality of the final separation is likely to be determined by the separation distance, OPLC and, for the preassay, the unsaturated S chamber must be selected. If RPC equipment is available for improving the efficiency of the final separation, the choice of chromatographic tank for the preassay depends on the types of compound to be separated. If the acidic or basic character of the vapour phase is important for the separation, a saturated S-chamber (microchamber) should be used; if this is not available, a saturated N chamber is the right selection for the TLC pre-assay. If the mobile phase is to be transferred to a U-RPC separation, an unsaturated S chamber (ultramicro chamber) must be chosen. These considerations are summarized in **Figure 5**.

Selection of Suitable Solvents

The modern strategy of solvent selection is based on the solvent classification by Snyder, who classified more than 80 solvents into eight groups for normal-phase chromatography according to their properties as proton acceptors (x_a) and proton donors (x_d), and their dipole–dipole interactions (x_n).

For, selection of suitable solvents, preliminary experiments are performed on silica TLC plates with the nine solvents indicated by stars in **Table 1**, which lists the solvents commonly used in planar chromatography.

Table 1 Solvent classification based on solvent strength and selectivity values

Group	*Solvent*	*Solvent strength* (S_i)	X_e	X_d	$S_v = \frac{X_e}{X_d}$
–	*n*-Hexane	0	–	–	0.10*
I	*n*-Butyl ether	2.1	0.44	0.18	2.44
	Diisopropyl ether	2.4	0.48	0.14	3.43
	Methyl-*t*-butyl ether	2.7	0.49	0.14	3.50
	Diethyl ether*	2.8	0.53	0.13	4.08
II	*i*-Pentanol	3.7	0.56	0.19	2.95
	n-Butanol	3.9	0.56	0.19	2.95
	i-Propanol	3.9	0.55	0.19	2.89
	n-Propanol	4.0	0.54	0.19	2.84
	Ethanol*	4.3	0.52	0.19	2.74
	Methanol	5.1	0.48	0.22	2.18
III	Tetrahydrofuran*	4.0	0.38	0.20	1.90
	Pyridine	5.3	0.41	0.22	1.86
	Methoxyethanol	5.5	0.38	0.24	1.58
	Methylformamide	6.0	0.41	0.23	1.78
	Dimethylformamide	6.4	0.39	0.21	1.86
	Dimethylsulfoxide	7.2	0.39	0.23	1.70
IV	Acetic acid*	6.0	0.39	0.31	1.26
	Formamide	9.6	0.36	0.23	1.57
V	Dichloromethane*	3.1	0.29	0.18	1.61
	1,1-Dichloroethane	3.5	0.30	0.21	1.43
	Benzyl alcohol	5.7	0.40	0.30	1.33
VI	Ethyl acetate*	4.4	0.34	0.23	1.48
	Methyl ethyl ketone	4.7	0.35	0.22	1.59
	Dioxane	4.8	0.36	0.24	1.50
	Acetone	5.1	0.35	0.23	1.52
	Acetonitrile	5.8	0.31	0.27	1.15
VII	Toluene*	2.4	0.25	0.28	0.89
	Benzene	2.7	0.23	0.32	0.72
	Nitrobenzene	4.4	0.26	0.30	0.87
	Nitromethane	6.0	0.28	0.31	0.90
VIII	Chloroform*	4.1	0.25	0.41	0.61
	Dodecafluoroheptanol	8.8	0.33	0.40	0.83
	Water	10.2	0.37	0.37	1.00

*Approximate value.

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After these initial TLC experiments with the neat solvents, the solvent strength (S_i) must either be reduced or increased so that the substance zones are distributed between hR_F 20 and 80. The two theoretical situations are depicted in **Figure 6** (A and P in **Figure 6**). If the compounds to be separated migrate in the upper third of the plate (A-a in **Figure 6**) the solvent strength must be reduced by dilution with hexane. If the neat solvents do not cause migration of the substances, the solvent strength must be increased (P-a in **Figure 6**) by the addition of water. In both circumstances the solvent strength should be varied so that better distribution of the substance zones is obtained. Consequently, the structures and properties of the compounds to be separated do not have to be known. Their classification as apolar (A) or polar (P) compounds can be made in accordance with their behaviour in these TLC experiments.

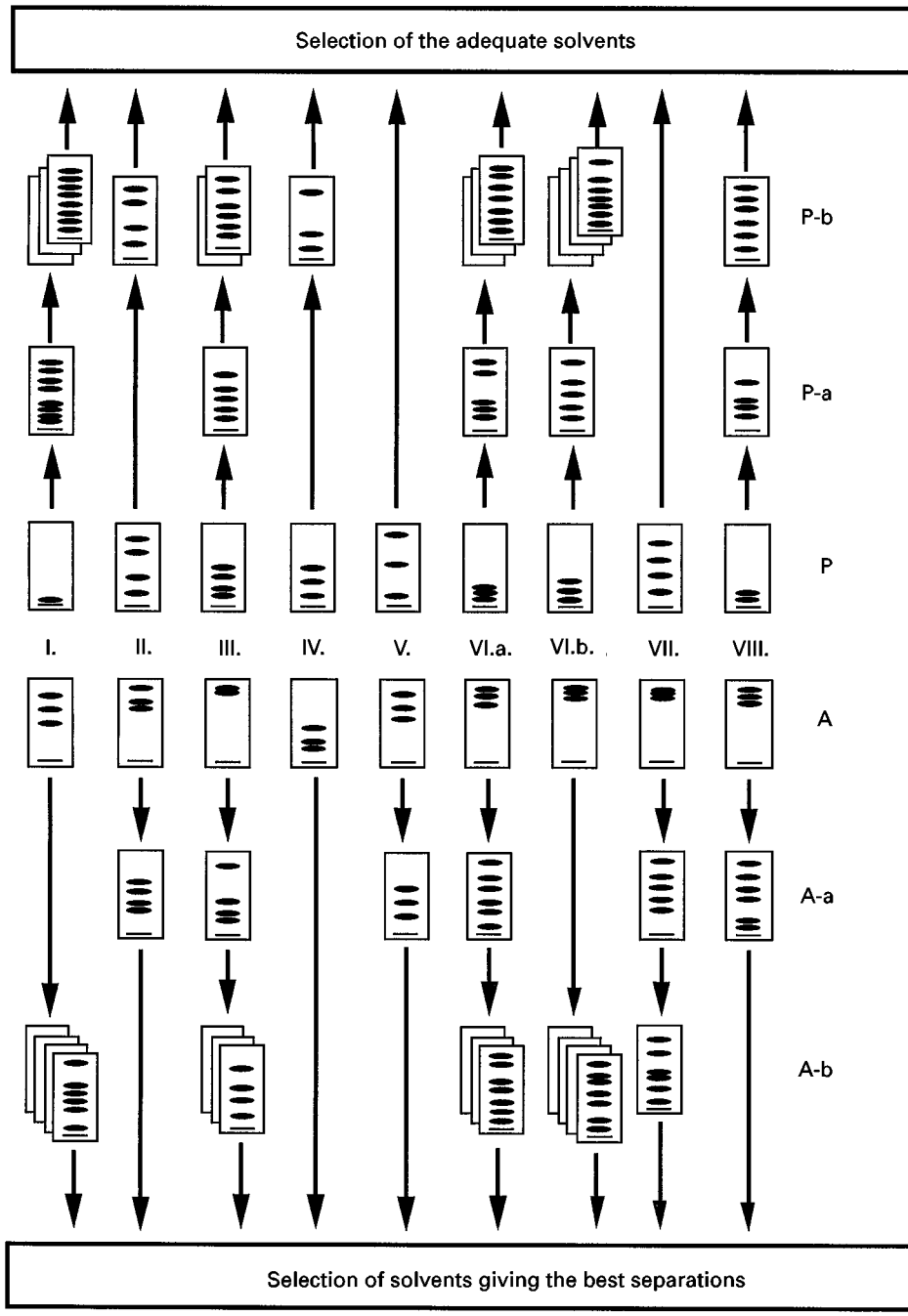

Figure 6 Strategy for the selection of a suitable TLC solvent.

If solvents result in good separation, their homologues or other solvents of the same group can also be tested, as indicated by A-b and P-b in **Figure 6**. After these experiments the solvents giving the best separations are chosen for further optimization of the separation of apolar compounds. For optimization of the mobile phase for separation of polar compounds, suitable solvents are again selected; the solvent mixture should contain one solvent in which the compounds do not migrate; this is necessary for the transfer of the mobile phase to certain forced-flow techniques. In certain circumstances a suitable separation can be achieved with this solvent-selection strategy. The individual steps of this method of solvent selection are depicted in a flow chart in **Figure 7**.

Thus the structures and properties of the compounds to be separated do not have to be known for these experiments. After these experiments, the solvents giving adequate separations are chosen for optimization of the mobile phase.

Mobile Phase Optimization

Mobile phase optimization is based both on modification of published data, on experience with the analytes, and on intuition. As sample composition becomes more complex, however, systematic solvent optimization becomes increasingly important. For systematic mobile phase optimization four methods are generally used in planar chromatography:

- window diagram
- sequential simplex method
- Geiss's structural approach
- the 'PRISMA' model.

Because only the 'PRISMA' model is currently suitable for both manual and automatic mobile phase optimization, this method is summarized below.

After the selection of suitable solvents the construction of the actual 'PRISMA' model is begun. In general between two and five solvents might be selected for the construction of the model; modifiers might

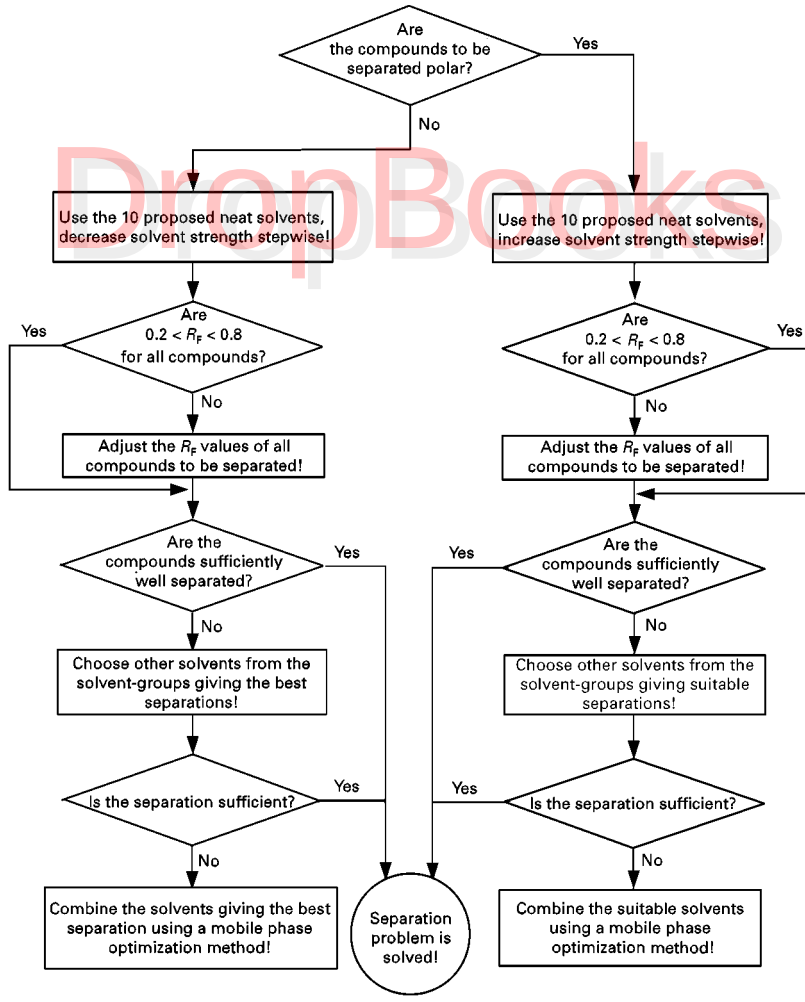
Are the compounds to be separated polar?
Yes
No
Use the 10 proposed neat solvents, decrease solvent strength stepwise!
Use the 10 proposed neat solvents, increase solvent strength stepwise!
Are 0.2 < R_F < 0.8 for all compounds?
Yes
No
Adjust the R_F values of all compounds to be separated!
Are the compounds sufficiently well separated?
Yes
No
Choose other solvents from the solvent-groups giving the best separations!
Choose other solvents from the solvent-groups giving suitable separations!
Is the separation sufficient?
Yes
No
Combine the solvents giving the best separation using a mobile phase optimization method!
Separation problem is solved!
Combine the suitable solvents using a mobile phase optimization method!

Figure 7 Flow chart illustrating a systematic approach for the selection of suitable solvents.

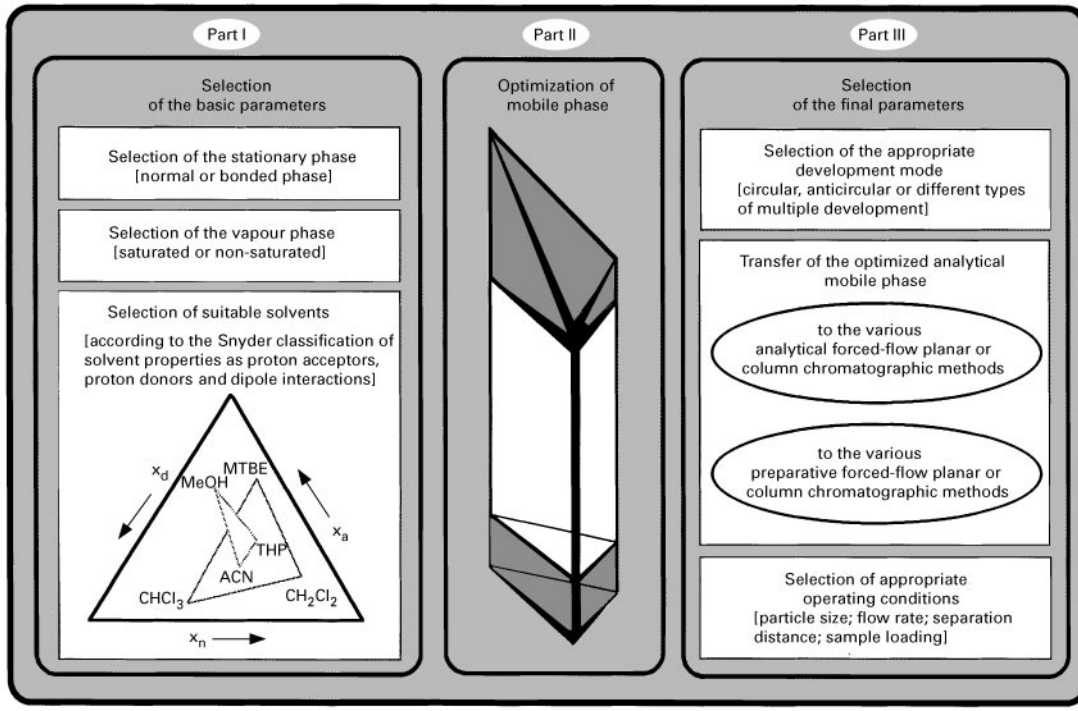
Part I
Part II
Part III
Selection of the basic parameters
Optimization of mobile phase
Selection of the final parameters
Selection of the stationary phase [normal or bonded phase]
Selection of the vapour phase [saturated or non-saturated]
Selection of suitable solvents
[according to the Snyder classification of solvent properties as proton acceptors, proton donors and dipole interactions]
MeOH
MTBE
THP
ACN
CHCl3
CH2Cl2
xd
xa
xn
Selection of the appropriate development mode [circular, anticircular or different types of multiple development]
Transfer of the optimized analytical mobile phase
to the various analytical forced-flow planar or column chromatographic methods
to the various preparative forced-flow planar or column chromatographic methods
Selection of appropriate operating conditions [particle size; flow rate; separation distance; sample loading]

Figure 8 The 'PRISMA' system for the systematic optimization of a planar chromatographic method.

also be added. The actual 'PRISMA' model is a three dimensional geometrical design which correlates the solvent strength with the selectivity value of the mobile phase. The tripartite model (see the central part of **Figure 8**) consists of an irregular top part (light grey), a regular middle part (white) and the lower part (dark grey) symbolizing the modifier(s). When working with silica as the stationary phase, the upper frustum is generally used for the optimization of mobile phases, with or without modifier, for the separation of polar compounds. The regular centre portion of the prism is used for the optimization of mobile phases, with or without modifier, for the separation of apolar compounds. The construction of the model, the role of solvent strength, and the characterization of the selectivity points (P_S) are described extensively in the literature.

The selectivity points on the vertical planes of the regular part of the prism can be obtained by diluting the solvent mixtures with a solvent-strength regulator. Solvent-strength (S_T) values decrease from top to bottom; at the base of the prism S_T is zero. If sections are taken across the regular prism parallel to the base, triangles of different S_T levels are obtained. Obviously, the solvent strength is identical at all points on one of these triangles, and all points on a vertical straight line correspond to the same selectivity point.

For normal-phase chromatography hexane ($S_i = 0$) is the regulator. If reversed-phase plates must be used for the separation, the regular part of the model is used for the separation, irrespective of the polarity of the compounds to be separated. In these circumstances water, rather than hexane, must be used as the solvent-strength regulator.

The solvent-strength values of the modifier(s) are treated by the 'PRISMA' model as additive terms. For the sake of simplicity, the solvent-strength values of the modifiers are neglected, because they are usually present at low, constant concentrations (generally between 0.1 and 3%, e.g. acids, ion pairs).

Manual Optimization Procedure

The four basic selectivity points within the regular part of the prism ($P_S = 333, 811, 181, 118$) for four solvent mixtures and the three basic selectivity points on the side of the prism ($P_S = 550, 75–25, 25–75$) for three solvent mixtures are emphasized in **Figure 9**. The black points symbolize mixtures of one solvent and the solvent strength regulator (binary systems); the dark grey points symbolize mixtures of two solvents and the regulator (ternary systems); and the three-digit numbers symbolize mixtures of three solvents and the regulator (quaternary systems).

If three solvents were selected for the separation of apolar compounds, optimization is performed within the regular part of the model with the help of the four basic selectivity points. The steps for optimizing the solvent combination for apolar compounds are

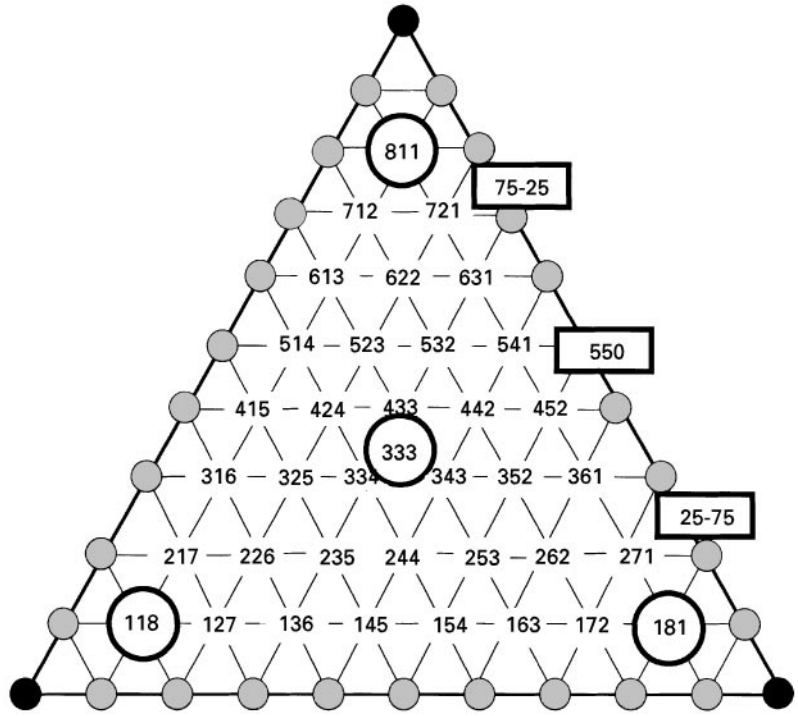

Figure 9 Favoured selectivity points for mobile phase optimization.

depicted in a flow chart in **Figure 10**. If two solvents were selected, the optimization is performed along the side of the prism. In both circumstances the solvent strength is adjusted and then different selectivity points are tested. If three to five solvents are selected as best, the number of solvents is reduced on the basis of criteria such as the number of compounds separated and the ΔR_F values obtained. If the solvent combinations tested with this strategy do not result in a sufficient separation, or at least the beginnings of a separation, of important pairs of substances, other solvents must be selected and the process must be repeated, as indicated in the flow chart.

For the separation of apolar compounds the optimization is generally a rapid process because a few experiments are sufficient to evaluate the optimum mobile-phase composition.

For polar compounds, the optimization is always started on the top irregular triangle of the model, either within the triangle, when three solvents were selected, or along one side, when two solvents were selected. Water is usually used as a modifier to increase solvent strength and reduce tailing; if water is used, several selectivity points cannot be tested because of immiscibility problems (especially near $P_S = 811$).

Changing the selectivity points on the top triangle also changes the solvent strength; thus a small change in the selectivity point might result in a large difference in resolution, especially when the solvent strength of the selected solvents differs substantially. The subsequent procedure is similar to that for the apolar compounds, but the solvent strength must be adjusted after a suitable selectivity is found. The flow chart for the optimization of the solvent combination for polar compounds is shown in **Figure 11**.

In contrast to the separation of apolar compounds, optimization is a longer process for polar substances because of the simultaneous change in solvent strength and selectivity. When water, in particular, is one of the solvents selected for the construction of the triangle, a small change in selectivity results in extreme changes in resolution. More chromatographic experience is, therefore, necessary if the separation problem is to be solved rapidly.

Manual optimization of the mobile phase must be performed until at least the beginnings of a separation of the compounds is obtained. This can usually be achieved with the first 'PRISMA' combination, assuming the individual solvents were selected correctly.

Automatic Optimization Procedure

The basis of automatic mobile-phase optimization, the correlation between mobile-phase composition and resolution for saturated TLC systems, can be described by mathematical functions. The correlation between hR_F values and the selectivity points at a constant solvent strength level can be expressed by

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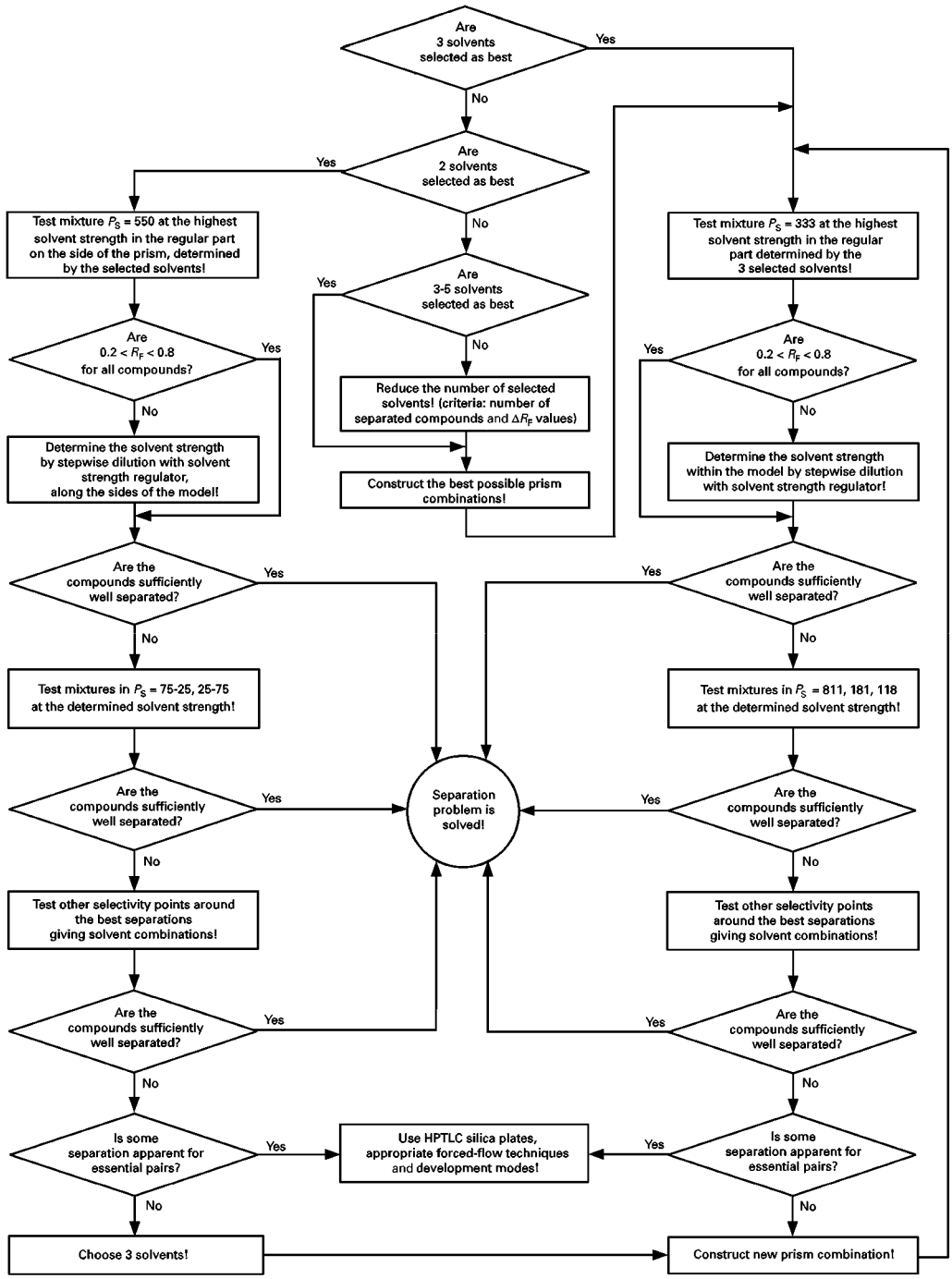
Are 3 solvents selected as best
Yes
No
Are 2 solvents selected as best
Yes
No
Are 3-5 solvents selected as best
Yes
No
Test mixture P_S = 550 at the highest solvent strength in the regular part on the side of the prism, determined by the selected solvents!
Reduce the number of selected solvents! (criteria: number of separated compounds and ΔR_F values)
Construct the best possible prism combinations!
Test mixture P_S = 333 at the highest solvent strength in the regular part determined by the 3 selected solvents!
Are $0.2 < R_F < 0.8$ for all compounds?
Yes
No
Determine the solvent strength by stepwise dilution with solvent strength regulator, along the sides of the model!
Determine the solvent strength within the model by stepwise dilution with solvent strength regulator!
Are the compounds sufficiently well separated?
Test mixtures in P_S = 75-25, 25-75 at the determined solvent strength!
Test mixtures in P_S = 811, 181, 118 at the determined solvent strength!
Separation problem is solved!
Test other selectivity points around the best separations giving solvent combinations!
Is some separation apparent for essential pairs?
Use HPTLC silica plates, appropriate forced-flow techniques and development modes!
Choose 3 solvents!
Construct new prism combination!

Figure 10 Flow chart illustrating a systematic approach for the optimization of the mobile phase for the separation of nonplanar compounds.

the function:

$$hR_F = a(P_S)^2 + b(P_S) + c$$

For quaternary solvent systems, the correlation between hR_F values and solvent strength at a constant selectivity point can be expressed by the function:

$$\ln hR_F = d(S_T) + e$$

Because the vertical correlation can be linearized, measurements on three solvent-strength levels are needed to calculate the hR_F values for all selectivity points in the spatial design. These correlations are also relevant when modifiers are used in constant amounts, for different classes of substance. From these correlations of hR_F values with the selectivity of the mobile phase, the chromatographic behaviour of

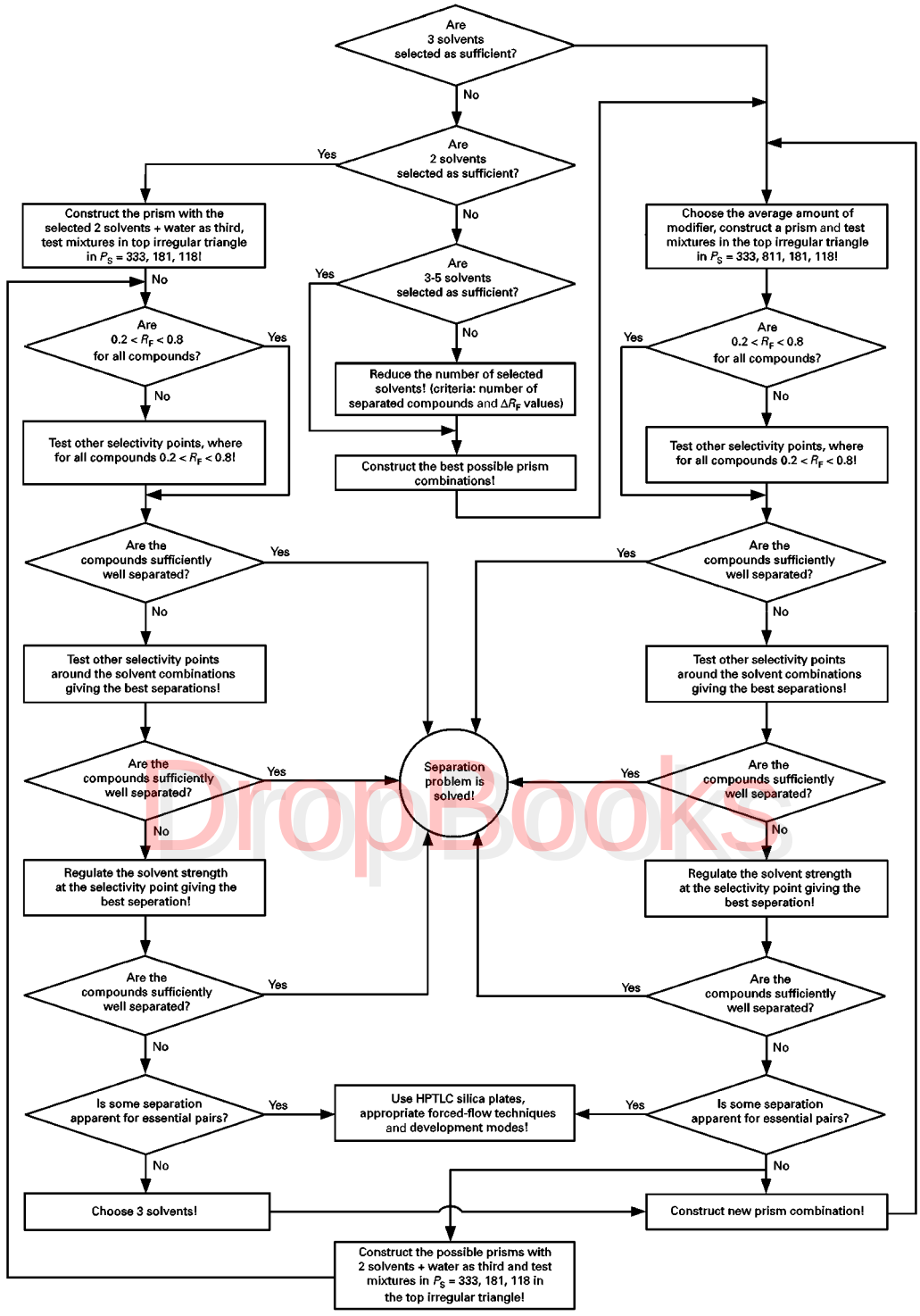
Are 3 solvents selected as sufficient?
No
Are 2 solvents selected as sufficient?
Yes
No
Are 3-5 solvents selected as sufficient?
Yes
No
Reduce the number of selected solvents! (criteria: number of separated compounds and ΔR_F values)
Construct the best possible prism combinations!
Construct the prism with the selected 2 solvents + water as third, test mixtures in top irregular triangle in P_S = 333, 181, 118!
Choose the average amount of modifier, construct a prism and test mixtures in the top irregular triangle in P_S = 333, 811, 181, 118!
Are $0.2 < R_F < 0.8$ for all compounds?
Test other selectivity points, where for all compounds $0.2 < R_F < 0.8$!
Are the compounds sufficiently well separated?
Test other selectivity points around the solvent combinations giving the best separations!
Separation problem is solved!
Regulate the solvent strength at the selectivity point giving the best seperation!
Is some separation apparent for essential pairs?
Use HPTLC silica plates, appropriate forced-flow techniques and development modes!
Choose 3 solvents!
Construct new prism combination!
Construct the possible prisms with 2 solvents + water as third and test mixtures in P_S = 333, 181, 118 in the top irregular triangle!
Yes
No

Figure 11 Flow chart illustrating a systematic approach for the optimization of the mobile phase for the separation of polar compounds.

substances to be separated can be predicted for all selectivity values in saturated chromatographic chambers.

The separation quality of predicted chromatograms can be assessed by use of a chromatographic response function (CRF). The optimum composition can be found by a simple mathematical procedure which maximizes the CRF by monitoring its dependence upon mobile-phase composition. Twelve measurements are necessary to discover a local opti-

Table 2 Required measurements for automatic mobile phase optimization to achieve the global optimum

Solvent strength	*Selectivity points*					
S_{T1}	811	631	118	343	136	181
S_{T2}	811	433	118	316	361	181
S_{T3}	811	613	118	334	163	181

mum, and fifteen for the global optimum. To increase the accuracy, six measurements at three different solvent strength levels (18 experiments) are necessary, as is seen in **Table 2**.

Selection of the Mode of Development

Planar chromatography differs from all other chromatographic methods in that it enables selection of the optimum mode of development; the linear mode of development is used most frequently. Because ascending development has no theoretical advantage over horizontal development, the latter, being more adaptable, has become increasingly common in recent years.

The advantage of circular development, where the solvent system migrates radially from the centre of the plate to the periphery, is well known for the separation of compounds in the lower R_F range. Working with the same mobile phase, the resolution is about 4–5 times higher in circular than in linear development mode, as is seen in **Figure 12**. This statement is only valid if the samples are spotted exactly at the centre ($x = 0$ cm). If the distance between the sample and the mobile phase inlet is, e.g. 2 cm, there is no significant difference in the lower R_F range between circular and linear development (see **Figure 12**). Development can, however, be started at a point displaced from the centre if a filter-paper ring is used to achieve higher mobile-phase velocity. Under these conditions many samples can be applied and the advantages of circular development can be exploited.

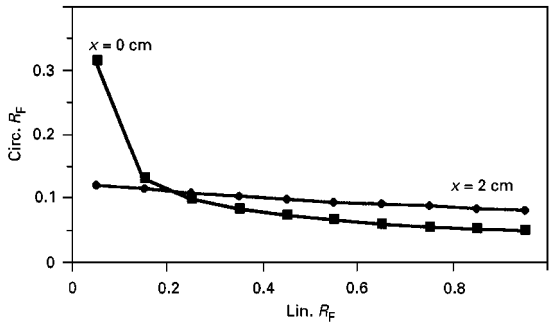

Figure 12 Effect on the ΔR_F value of the distance between mobile phase inlet and sample.

In anticircular development the mobile phase is applied to the layer as a circle and flows towards the centre. Because the solvent flow velocity decreases with the square of the distance, but the area wetted also decreases with the square of the distance travelled, the speed of mobile-phase migration is practically constant. Although anticircular development is rarely used, it is an accepted approach in TLC if resolution must be increased in the higher R_F range.

The multiple development (MD) techniques, UMD (unidimensional MD) and IMD (incremental MD) can also be used to increase separating power in the lower R_F range. UMD is the repeated development of the plate over the same development distance with mobile phase of the same composition; between development steps the mobile phase is removed from the layer by careful drying and the dried plate is returned to the development chamber for development under the same chromatographic conditions as previously. IMD is an alternative version of this technique in which successive chromatographic developments are performed over increasing development distances with mobile phase of the same composition. In the IMD first development distance is the shortest and subsequent development steps are over longer distances; the development distance usually increases by equal increments. The last migration distance, the longest, corresponds to the useful development length of the plate (but can depend on the mobile phase employed).

The advantages of the different modes of development can be summarized as follows:

- circular development increases resolution in the lower R_F range
- anticircular development increases resolution in the higher R_F range
- UMD is most effective at improving separation in the lower R_F range
- IMD improves zone-centre separation.

A comparison of these modes of development is presented in **Figure 13**.

Mobile Phase Transfer

There are two reasons for transferring the optimized TLC mobile phase. The first is that the separation is not sufficiently good and better resolution might be achieved by use of forced-flow methods. The optimized TLC mobile phase is, therefore, transferred without alteration to the U-RPC or OPLC technique. When the latter is used, a prerun must be performed. For separation of nonpolar compounds the prerun can be performed with hexane; for separation of polar

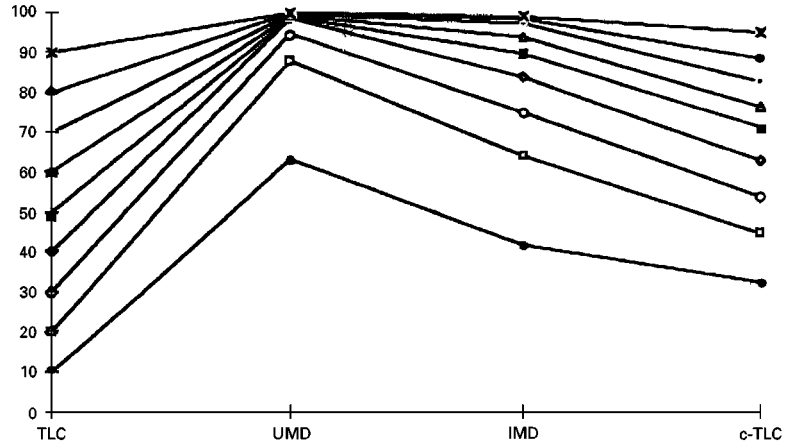

Figure 13 Effect of linear, circular, UMD, and IMD development modes on R_F values in the lower R_F range.

substances the prerun can be performed with any component of the mobile phase in which the components do not migrate. The selection of this solvent might be considered during optimization of the mobile phase. Highly effective separation can be achieved by use of HPTLC plates and forced-flow techniques.

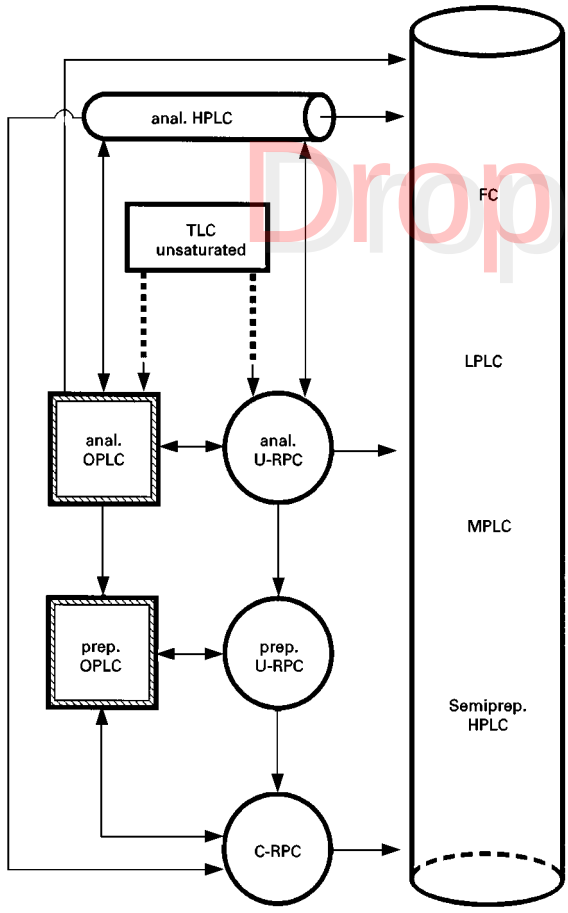

Figure 14 Possibilities of transferring the optimized TLC mobile phase to the different forced-flow planar chromatographic methods, and to preparative column liquid chromatographic techniques.

The second reason for transferring an optimized TLC mobile phase is when scaling up to the various preparative chromatographic systems. As a result of the characterization of the different saturation grade of chromatographic chambers (see **Figure 4**), excellent mobile phase transfer between analytical and preparative planar chromatographic methods and analytical HPLC can be achieved. The transfer can be performed on the basis of the chromatographic conditions used. Dry-filled preparative columns (for flash, low-pressure liquid, and medium-pressure liquid chromatography) can be equilibrated with the solvent used for the prerun in analytical OPLC, whereas if the column is filled by the slurry technique, the slurry must be prepared from the same solvent as was used for the OPLC prerun. In both of these, air bubbles can be eliminated by passage of an appropriate amount of the solvent used for the prerun; preparative separation can then be started with the optimized unsaturated TLC mobile phase.

The possibilities of mobile-phase transfer between the different solid–liquid chromatographic methods are comprehensively summarized in **Figure 14**, which demonstrates the possibilities of direct transfer. Different lines show those applicable to the different methods; dotted lines and thin lines are indicative of offline and online methods, respectively, whereas thick lines indicate the possibility of transfer of the optimized mobile phase without change between different solid–liquid planar and column chromatographic techniques, both offline and online.

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Selection of Other Operating Parameters

In conventional TLC the solvent velocity cannot, in principle, be influenced by the chromatographer. The enhanced efficiency of forced-flow techniques, compared with TLC driven by capillary action only, results from the constant linear mobile phase velocity. Forced-flow techniques guarantee optimum H/u values. In OPLC the upper limit of velocity depends on the applied external pressure and on the viscosity. In RPC, the greater the speed of rotation, the faster

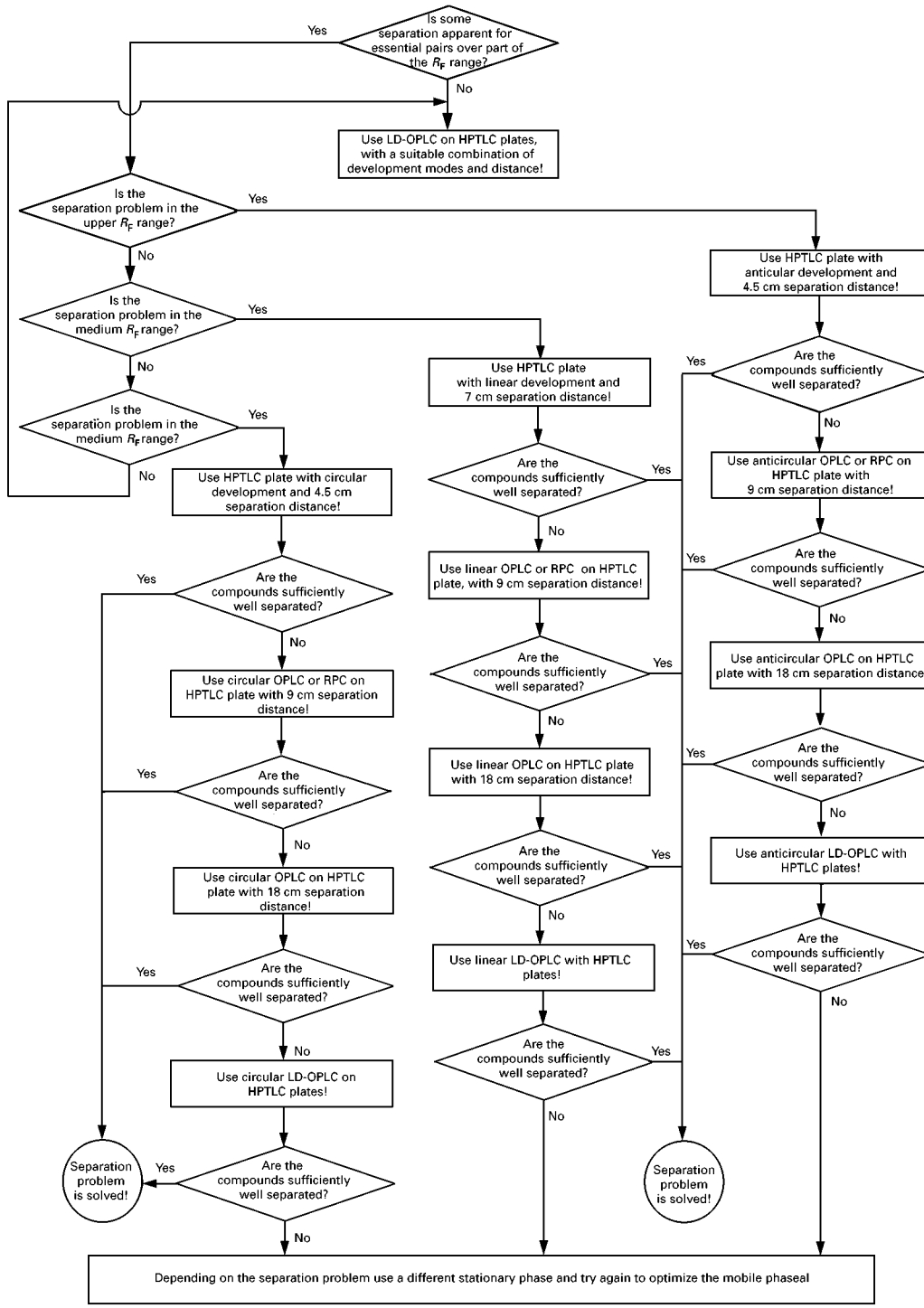
Is some separation apparent for essential pairs over part of the R_F range?
Yes
No
Use LD-OPLC on HPTLC plates, with a suitable combination of development modes and distance!
Is the separation problem in the upper R_F range?
Yes
No
Use HPTLC plate with anticular development and 4.5 cm separation distance!
Is the separation problem in the medium R_F range?
Yes
No
Use HPTLC plate with linear development and 7 cm separation distance!
Are the compounds sufficiently well separated?
Yes
No
Use anticircular OPLC or RPC on HPTLC plate with 9 cm separation distance!
Use anticircular OPLC on HPTLC plate with 18 cm separation distance!
Use anticircular LD-OPLC with HPTLC plates!
Is the separation problem in the medium R_F range?
Yes
No
Use HPTLC plate with circular development and 4.5 cm separation distance!
Use linear OPLC or RPC on HPTLC plate, with 9 cm separation distance!
Use linear OPLC on HPTLC plate with 18 cm separation distance!
Use linear LD-OPLC with HPTLC plates!
Use circular OPLC or RPC on HPTLC plate with 9 cm separation distance!
Use circular OPLC on HPTLC plate with 18 cm separation distance!
Use circular LD-OPLC on HPTLC plates!
Separation problem is solved!
Depending on the separation problem use a different stationary phase and try again to optimize the mobile phaseal

Figure 15 Flow chart illustrating a systematic approach for the selection of the mode of development, development distance, and forced-flow technique.

the migration of the mobile phase. The local mobile-phase velocity can be influenced by the mode of development selected.

In TLC separation efficiency improves with the square root of the separation distance. The optimum, however, depends on the quality of the plate (average particle size and size distribution of the stationary phase), the vapour space, the mode of development, and the properties of the compounds to be separated. The first of these cannot be influenced by the user of precoated plates. The maximum length of commercially available precoated plates is 20 cm. Thus, the maximum separation distance in linear development is 18 cm. The efficiency and rapidity of planar chromatography can be increased by the use of a novel category of multilayer OPLC, long-distance OPLC, by use of which the separation efficiency is increased significantly. In this technique the end of the first plate has a slit-like perforation through which the mobile phase is transferred to a second layer. Clearly, on this basis, a very long separation distance can be achieved by combining one plate with another.

Sample application is one of the most important stages of successful planar chromatography. The amount of applied sample depends on the determination method. Generally, μg and ng quantities of sample can be determined, but even less than 100 pmol substance per chromatogram zone has been reported.

During method development, the separation distance always depends on the mode of development and the forced-flow technique used, and on the development distance; this is summarized in **Figure 15** in the form of a flow-chart.

In normal circumstances alteration of temperature is not an effective means of modifying selectivity and maximizing resolution. If two compounds are unresolved at a given temperature, they normally remain unseparated at other temperatures, irrespective of whether N- or S-chambers are used. It can generally be stated that in saturated chromatographic chambers, which are most commonly used, the temperature does not have a great influence on separations. A change of $\pm 5^{\circ}C$ results in a change in hR_F of less than 3. Nevertheless, in the interest of reproducibility in duplicate separations it is important to note the working temperature. Remarkably, temperature is now being found to play an important role in the selectivity and efficiency of OPLC separations.

Strategy of Method Development

The 'PRISMA' optimization system is a strategy for method development in liquid chromatography. Figure 8, which shows the 'PRISMA' system for planar chromatography, consists of three parts. The first part is the selection of the basic parameters; stationary and vapour phases and suitable solvents, the last according to the Snyder classification. The second part is the optimization of the mobile phase, using the 'PRISMA' optimization model. The third part is the selection of the final parameters; the mode of development, transfer of the mobile phase to the appropriate forced-flow method and, last but not least, the selection of suitable operating conditions. The 'PRISMA' system enables the combination of the appropriate mode of development with the appropriate forced-flow technique by the use of a mobile phase of optimized composition; this offers special possibilities for solving difficult separation problems. This system provides guidelines for method development in planar chromatography.

See also: **II/Chromatography: Thin-Layer (Planar) Chromatography:** Historical Development; Instrumentation; Layers; Modes of Development; Conventional; Modes of Development; Forced Flow Overpressured Layer and Centrifugal Chromatography.

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